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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

NON-TARGET PLANTS:

SEED GERMINATION/SEEDLING EMERGENCE -

TIERS 1 AND 2

Prepared by

Robert W. Holst, Ph.D.

Standard Evaluation Procedures Project Manager
Stephen L. Johnson
Hazard Evaluation Division
Office of Pesticide Programs

United States Environmental Protection Agency
Office of Pesticide Programs
Washington, D.C. 20460

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The Standard Evaluation Procedure for Seed Germination/Seedling Emergence, Tiers 1 and 2, a Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Standard Evaluation Procedure (SEP), prescribes the evaluation criteria and procedures to assess this pesticide phytotoxicity study that the U.S. Environmental Protection Agency recommends to support the registration of manufacturing-use and formulated end-use products. This SEP, in conjunction with the Pesticide Assessment Guidelines, Subdivision J, is designed to aid data reviewers in theri evaluations of the seed germination/seedling emergence studies submitted by registrants. A summary of the rulemaking phytotoxicity test requirements can be found in 40 CFR Part 158. The study results contained in the evaluation procedure together with environmental fate and efficacy data are used to assess the potential hazard of pesticides to terrestrial and aquatic nontarget plants.

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NON-TARGET PLANTS:

SEED GERMINATION/SEEDLING EMERGENCE - TIERS 1 AND 2

I. INTRODUCTION

A. Purpose of the Standard Evaluation Procedure

This Standard Evaluation Procedure is designed to aid Ecological Effects Branch (EEB) data reviewers in their evaluations of preliminary (Tier 1) laboratory seed germination/seedling emergence studies submitted by registrants in the assessment of pesticide effects on non-target plants. This document is also designed to aid EEB reviewers in their evaluations of laboratory/greenhouse/small field plot (Tier 2) seed germination/seedling emergence studies submitted by registrants for the same purpose.

B. Background Information

Seed germination/seedling emergence studies are designed to provide phytotoxicity data on a pesticide. These phytotoxicity data are needed to evaluate the effect of the level of pesticide exposure to non-target and terrestrial plants and to assess the impact of pesticides on endangered and threatened plants as noted under the Endangered Species Act. The preliminary level (Tier 1) study evaluates the effect of the maximum exposure level while the greenhouse/laboratory/small field plot (Tier 2) study evaluates the effects of differing exposure levels. Where a phytotoxic effect is noted in one or more plants, further seed germination/seedling emergence studies may be required. These studies are required by 40 CFR § 158.150 to support the registration of any pesticide intended for outdoor use under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended.

Pesticides with outdoor use patterns that do not readily release the pesticide to the environment do not have to be evaluated using this phytotoxicity test. These use patterns include tree injection, subsurface soil applications, recapture systems, wick applications, and swimming pool uses. If any of these use patterns do readily expose non-target plants to the pesticide, as through vapors, the pesticide phytotoxicity potential may need to be evaluated.

C. Objective of Seed Germination/Seedling Emergence Tests

1. Tier 1 Test

The objective of the Tier 1 seed germination/seedling emergence test is to determine if a pesticide exerts a detrimental effect to plants during critical stages in their development. The test is performed on species from a cross-section of the non-target terrestrial plant population that have been historically used for this type

of testing and, therefore, have known types of responses. This is a maximum dose test designed to quickly evaluate the phytotoxic effects of the pesticide at the one dose.

2. Tier 2 Test

The objective of the Tier 2 seed germination/seedling emergence test is to determine if a pesticide exerts a detrimental effect to plants during critical stages in their development. The test is performed on species from a cross-section of the non-target terrestrial plant population that have been historically used for this type of testing and, therefore, have known types of responses. This is a multiple dose test designed to evaluate the phytotoxic effects of the pesticide over a wide range of anticipated pesticide quantities as may be found in the environment.

II. INFORMATION TO BE SUPPLIED

The registrant's report on preliminary seed germination/seedling emergence studies should include all information necessary to provide: 1) a complete and accurate description of the laboratory/greenhouse treatments and procedures, 2) sampling data and phytotoxicity rating, 3) data on storage of the plant materials until analysis, if so performed, 4) any chemical analysis of the plant material as to chemical content, if so performed, 5) reporting of the data, rating system and statistical analysis, and 6) quality control measures/precautions taken to ensure the fidelity of the operations.

A guideline of specific information that should be included in the registrant's report on seed germination/seedling emergence studies is provided in Appendix 1 of this document. The lists of requested information and reviewer aids are derived from the Pesticide Assessment Guidelines, Subdivision J: Hazard Evaluation of Non-Target Plants, which is complemented by this Standard Evaluation Procedure.

III. DATA INTERPRETATION

The acceptability of the study results will depend upon whether the test requirements/standards are followed. If a deviation is made, a determination must be made as to whether the deviation has changed the quality of the results in such a manner that the results cannot be extrapolated to the natural environment. There should be little or no deviation from the liberal standards prescribed in this study.

The results of the pesticide phytotoxicity tests with respect to the quantity of material applied to or near the seed are important. The concentration of the chemical in the carrier is important in that even slightly stronger concentrations than normally used can lead to

stunting and necrosis. Subtoxic concentrations, on the other hand, may cause unwanted rapid growth.

Plants can recover from certain types of injury with little or no resulting effect on the esthetic or economic value of the plant(s) tested or upon which an evaluation is made. Therefore, it is important that a minimum of two weeks of observations be made after application of the pesticide to evaluate seedling emergence. If seed germination is evaluated, the extent of germination (percentage of seed showing root and shoot emergence) should be evaluated at least five days after imbibition.

A decision point to proceed to the next higher test is a 25% detrimental effect, i.e., a 25% change in the average germination or plant growth or injury as compared to untreated controls. This level is considered to be that point at which the plants will not recover to their full esthetic value, economic value or reproductive potential as in the case of the maintenance of the endangered or threatened species.

IV. THE DATA EVALUATION PROCESS

Upon careful examination of the information/data supplied by the registrant in his submission to the Agency, the reviewer shall evaluate the data as follows.

A. Identify Data Gaps

Using Appendix 1 of this document as a guide, the reviewer should then look for data gaps - omissions in the information supplied by the registrant in his report. These should be duly noted in the reviewer's report, and a judgment made as to which are considered significant enough to adversely affect the review process. Those so identified should be communicated back to the registrant by the Product Manager for corrective action.

B. Assess the Appropriateness and Adequacy of the Data

The data reviewer then considers the appropriateness, i.e., the intended use pattern, and adequacy of the data/information that has been supplied. Appendix 1 of this document is a useful guide to the various parameters that need to be considered. Appendix 2 provides specific questions that should be answered by the reviewer during the study evaluation process. Statistical treatments of the data should be independently verified and the quality control precautions noted.

As an adjunct to these, the reviewer should draw upon the technical guidance in the reviewer aids materials that are available. (See also the recommended references in Subdivision J - Hazard Evaluation: Non-Target Plants.) A listing of additional source materials is located in the References section of this document.

In addition to the data gaps noted above, any perceived deficiencies in the data/information supplied should also be identified. A statement as to these deficiencies should be made in the reviewer's report and corrective action to resolve them should be provided. This information can be relayed to the registrant by the Product Manager for appropriate action.

C. Report Preparation

The Agency reviewer prepares a standard review report following the standard format for preparation of scientific reviews as provided in Appendix 3 of this document. All important information provided by the registrant including the methodology and results should be summarized in order that future evaluations can be made. The results may be expressed in the form of tables where specific values are related. Figures (graphs) may be provided but are not to be the sole source of the values needed for future evaluations.

D. Conclude if the Requested Action is Supportable

Lastly, the reviewer considers the results of the seed germination/seedling emergence studies and makes a judgment as to whether they support the requested registration action of the data submitter. If the data are not supportive, possible alternative action(s) that may be taken by the registrant, such as label modifications, are suggested. If deficiencies/omissions exist in the submitted data, the reviewer may have to defer judgment until such time as appropriate corrective action has been rendered by the registrant.

APPENDIX 1

INFORMATION REQUESTED OF THE REGISTRANT

The registrant's report on preliminary seed germination/seedling emergence studies should include all information necessary to provide: 1) a complete and accurate description of the laboratory/greenhouse/small field plot treatments and procedures, 2) sampling and phytotoxicity rating, 3) data on storage of the plant material until analyzed, if so performed, 4) any chemical analysis of the plant material as to chemical content, if so performed, 5) reporting of the data, rating system and statistical analysis, and 6) quality control measures/precautions taken to ensure the fidelity of the operations.

Specifically, each laboratory/greenhouse/small field plot seed germination/seedling emergence report should include the following information.

I. General

° Cooperator or researcher (name and address), test location (county and state; country, if outside of the U.S.A.), and date of study;

° Name (and signature), title, organization, address, and telephone number of the person(s) responsible for planning/supervising/monitoring and, for the field plot studies, applying the pesticide;

° Trial identification number;

° Quality assurance indicating: control measures/precautions followed to ensure the fidelity of the phytotoxicity determinations; record-keeping procedures and availability of logbooks; skill of the laboratory personnel; equipment status of the laboratory or greenhouse; degree of adherence to good laboratory practices; and degree of adherence to good agricultural practices in maintaining healthly plants; and

° Other information the registrant considers appropriate and relevant to provide a complete and thorough description of the test procedures and results.

II. Test Substance (Pesticide)

° Identification of the test pesticide active ingredient (ai) including chemical name, common name (ANSI, BSI, ISO, WSSA), and Company developmental/experimental name;

° Active ingredient percentage in the technical grade material or in the manufacturing-use product, if the technical grade material is unavailable for test purposes;

° Solvent used to dissolve and apply the pesticide if the pesticide is insoluble in water or other intended carrier;

° Dose rate(s) in terms of active ingredient per area of land or concentration as applied;

° For Tier 1, dose rate(s) in terms of the maximum label rate, or if the registrant has shown that the maximum quantity that will be present in the non-target area is significantly less than the maximum label rate, the dose equal to or no less than three times that maximum environmental quantity;

° For Tier 2, dose rate(s) in terms of less than the maximum label rate, with dosages in a geometrical progression of no more than two-fold and with subtoxic (< EC_{50} level) and non-toxic (no-observable-effect-level) concentrations;

° Method of application including equipment type; and

° Number of applications.

III. Plant Species

° For Tier 1, identification of the six dicotyledoneae species and four monocotyledoneae species with family identification. The six dicots are to be of at least four different families and the moncots of at least two families. Soybeans, corn, and a dicot root crop like carrot are the required species. The proposed species and families as originally provided in Subpart J of the proposed guidelines [FR notice of 3 November 1980] are given below and are acceptable for the laboratory/greenhouse seed germination/seedling emergence test:

Family	Species	Common
Solanaceae	Lycopersicon esculentum	Tomato
Cucurbitaceae	Cucumis sativus	Cucumber
Compositae	Lactuca sativa	Lettuce
Leguminosae	Glycine max (Innoculation with Rhizobium japonicum is unnecessary)	Soybean
Cruciferae	Brassica oleracea	Cabbage
Umbelliferae	Daucus carota	Carrot
Gramineae	Avena sativa	Oat
Gramineae	Lolium perenne	Perennial Ryegrass

Family	Species	Common
Gramineae	Zea mays	Corn
Amaryllidaceae	Allium cepa	Onion

Seeds of plants with a low or variable germination potential should be avoided for the seed germination study.

° For Tier 2, identification of the plant species tested including those phytotoxically affected in the Tier 1 test;

° Identification of the cultivar(s) of the plant species or assignment of an identification number to the cultivar used and seed or plant source;

° Identification of the number of replicates and the number of plants per replicate per dose; and

° Identification of the date of planting or imbibition, date of pesticide application, and date of phytotoxicity rating or harvest and analysis.

IV. Site of the Test

° Site description of the seed germination/seedling emergence study such as the type of growth chamber, greenhouse, or field (small field plots);

° Location of the test site;

° Climatological data during the test (records of applicable conditions for the type of site, i.e., temperature and thermoperiod, rainfall or watering regime, light regime - intensity and quality, relative humidity, wind speed);

° Field lay-out (for small field plots), e.g., size and number of control and experimental plots; number of plants per plot/unit area;

° Pot, plant or row density of seeds or plants;

° Cultural practices such as cultivation and irrigation; and

° Substrate characteristics (name/designation of soil type and its physical and chemical properties, including pH and percent organic matter).

V. Results

° Reporting of percent germination/emergence, root length or other growth parameters that may have been measured to ascertain

toxic effects of the pesticide upon the plants with dates of observations;

° Phytotoxicity rating (including a description of the rating system) for each plant or population in the test; and

° Statistical analysis of the results including an environmental or effective concentration (EC) value. (Note, for Tier 1, there will be only a percent effect level at a specific concentration which is then compared to 25% of the growth [mass or rate] of the control.)

VI. Evaluation

° For Tier 1 studies, determination as to whether Tier 2 studies would be required due to phytotoxic effects noted in one or more of the tested species.

° For Tier 2 studies, determination as to whether Tier 3 tests (terrestrial field study) would be required due to phytotoxic effects noted in one or more of the tested species.

APPENDIX 2

SPECIFIC QUESTIONS FOR THE REVIEWER

The following questions are provided to aid the reviewer in performing the standard evaluation procedure in a scientific manner and in acquiring the necessary information to complete a standard format for preparation of scientific reviews.

I. General

° Was the name of the cooperator or researcher (name and address), test location (county and state; country, if outside of the U.S.A.), and date of study provided?

° Was the name (and signature), title, organization, address, and telephone number of the person(s) responsible for planning/supervising/monitoring and, for small field plot studies, applying the pesticide provided?

° Was the trial identification number provided?

° Were quality assurance control measures/precautions indicated?

° Was the Tier 1 seed germination/seedling emergence study done as a separate study? If not, were the doses and plant species required by Tier 1 included in the Tier 2 study?

II. Test Chemical

° Is the test chemical being used the technical grade, or if not available, the manufacturing-use product with the highest percentage of active ingredient?

° Is the active ingredient percentage or degree of purity of the chemical given?

° If a solvent was used, was it used at concentrations that are not phytotoxic and was a solvent control used?

° Is the dose given in quantity per unit area (of plant or land surface) or in tank concentration?

° For Tier 1, was the dose equal to or greater than the maximum label rate, or if the registrant has shown that the maximum quantity that will be present in the non-target area is significantly less than the maximum label rate, was the dose equal to or no less than three times that maximum environmental quantity?

° For Tier 2, was the maximum dose less than the maximum label rate?

° For Tier 2, were the additional dosages of a geometric progression of no more than two-fold, e.g., 0.1, 0.2, 0.4, 0.8, 1.6 kg/ha?

° For Tier 2, were a subtoxic (< EC_{50} level) and a non-toxic (no-observable-effect-level) concentration evaluated?

III. Test Species

° For Tier 1, were at least ten different species tested with species names provided?

° For Tier 1, were the ten species split between monocots and dicots, four and six, respectively?

° For Tier 1, were the ten species from six different families and the family names provided?

° For Tier 1, were two of the species tested soybeans and corn and was the third species a dicot root crop?

° For Tier 2, were at least those species that were phytotoxically affected in Tier 1 tested?

° Where various cultivars could be used, such as in the case of most agronomic and horticultural plants, were cultivar or varietal names provided?

° Were seed and plant sources provided?

° Were at least three replicates used with ten seeds per replicate for each dose level?

° Were some of the seeds pretested for germination and emergence potential? Seeds of plants with a low or variable potential should be avoided.

° Were endangered or threatened plant species not used?

IV. Test Procedures

° Was the test site specified, i.e., greenhouse, growth chamber, or small field plot?

° Were the environmental conditions that prevailed during the test (temperature and thermoperiod, light regime - intensity and quality, rainfall or watering regime, relative humidity, wind) provided as appropriate for the site?

° Were the environmental conditions that prevailed during the test those most favorable and most typical to the growth of the plants used? Were these conditions referenced?

° Was the test duration for seedling emergence at least two weeks in length or for seed germination at least five days in length?

° Were observations taken at least weekly for seedling emergence and after the five days for seed germination?

° Was the method of pesticide application including the type of application equipment employed given?

V. Reporting

° Were the detrimental effects reported as severity of phytotoxicity (rating or percentage), percent germination or percent emergence?

° If a rating system was used, was an explanation provided?

° Were abnormal changes in growth, development and/or morphology reported with comparisons to the controls or "normal" plants?

° Though not required, were direct measurements of root length or seedling length provided?

° Were the results statistically analyzed? Note that care should be taken in interpreting the statistical results where the sample size is small.

VI. Evaluation

° Were the results tabulated to indicate a percentage effect level (EC value) for each species as compared to the untreated control plants?

° For Tier 1 studies, was a determination made as to whether Tier 2 tests should be performed if any of the Tier 1 species were detrimentally affected (greater than 25% detrimental effect on growth)?

° For Tier 2 studies, were 25 and 50 percent detrimental effect levels determined for those plant species of Tier 1 that showed a phytotoxic effect to the chemical?

° For Tier 2 studies, was a determination made as to whether Tier 3 tests (terrestrial field study) should be performed if any of the Tier 2 species were detrimentally affected (greater than 25% detrimental effect on growth)?

APPENDIX 3

SAMPLE STANDARD FORMAT FOR PREPARATION OF SCIENTIFIC REVIEWS

The following format shall be used in documenting the review of the Subdivision J - Hazard Evaluation: Non-Target Plants - Seed Germination/Seedling Emergence Tier 1 and Tier 2 Studies.

Chemical: (Common Name)

Formulation: (Percent Active Ingredient)

Study/Action: (Purpose of the Submission)

Study Identification:

(Subdivision J Test Title)
(Reference or Registrant Data Information with Study Number)
(EPA Accession Number)

Reviewer: (Name and Address of Reviewer; Date of Review)

Approval: (Quality Control Reviewer)

Conclusions: (Summary and Conclusion of Tests)

Acceptability and Recommendations:

(Decide as to (1) the scientific validity of the study and (2) compliance to the Subdivision J - Seed Germination Tier 1 and Tier 2 Studies.)

Background: (Introductory Information and Directions for Use)

Discussion:
1. Study Identification
2. Materials and Methods
3. Reported Results
4. Reported Conclusions
5. Reviewer's Interpretation of Results and Conclusion

REFERENCES

Bewley, J. D. 1983. Physiology and Biochemistry of Seeds in Relation to Germination.

Khan, A. A. 1977. Physiology and Biochemistry of Seed Dormancy and Germination.

Mayer, A. M. 1982. Germination of Seeds.

Truelove, B., ed. 1977. Research Methods in Weed Science. Southern Weed Science Society. Auburn, AL: Auburn Printing, Inc.

U.S. Department of Agriculture. 1952. Manual for Testing Agricultural and Vegetable Seeds. Agriculture Handbook No. 30.

Other scientific articles of seed germination may be found in the following journals:

Agronomy Journal
Environmental Science and Technology
Journal of Environmental Quality
Soil Science and Plant Nutrition
Weed Science